

The influence of oxygen availability on oxygen consumption in the freshwater clam *Musculium partumeium* (Say) (Bivalvia: Sphaeriidae)

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Abstract. The O₂ consumption of the freshwater clam *Musculium partumeium* Say was determined as a function of clam size and O₂ availability. The rate of O₂ consumption by large individuals was influenced significantly by O₂ availability whereas the rates of smaller individuals were not altered significantly. One reason for the difference in response for large and small animals was the greater degree of availability in O₂ consumption among smaller individuals.

A number of studies have examined the influence of low O₂ availability on metabolism in marine bivalves (e.g. Newell, 1979 and references therein). Much of this work is predicated upon the supposition that the tidal exposure of many bivalves in marine habitats regularly subjects them to periods of low O₂ availability and, thus, these forms could have responded evolutionarily to this selection pressure.

Few studies, however, have examined the influence of reduced O₂ availability in freshwater bivalves (Burky, 1983; Burky *et al.*, 1985; Hornbach, 1985). While there is not a cyclical tidal change in O₂ availability in freshwater systems, there are a number of factors which could affect O₂ accessibility. Diurnal rhythms of O₂ concentration in littoral areas are known to occur as a result of shifts in the dominance of photosynthetic or respiratory activities in these highly productive areas. Seasonal shifts in O₂ availability in the profundal zones of lakes associated with temperature stratification and subsequent turnover events are also well documented. Probably one of the most severe of freshwater habitats, in terms of changes in O₂ availability, are ephemeral ponds. In these habitats, O₂ levels can be high in the spring as the pond fills, but as water levels decline in the mid to late summer, O₂ availability often becomes restricted. Also, many ephemeral ponds have heavy loads of organic debris which can contribute to anoxic conditions in very shallow water during any season.

One of the most common inhabitants of ephemeral ponds are bivalves of the family Sphaeriidae, the fingernail and pea clams. Within this family, the genus *Musculium* has the largest population of species found in temporary habitats (Burch, 1975). A moderately large body of information is available concerning life history adaptations in this genus for survival in ephemeral ponds (see e.g. Hornbach and

Childers, 1986), yet little information is available concerning physiological adaptations for this harsh habitat (Burky, 1983). This study examines the influence of O₂ availability and body size on O₂ consumption in the clam, *Musculium partumeium* (Say).

MATERIALS AND METHODS

Clams utilized in this study were collected in May - July 1986 from a temporary pond located at the Kathryn Ordway Natural History Area in Inver Grove Heights, Minnesota. In all cases, clams were removed from the pond when sediment temperatures were near 17°C (range 16.5 - 18.0°C). Specimens were returned to the laboratory and maintained at 17°C in filtered pond water (Whatman "42", effective pore size 2.5 µm) for 6-12 hr before metabolic rates were determined. The shell length (anterior-posterior dimension) was measured to the nearest 0.1 mm with a dissecting microscope fitted with a stage mounted micrometer.

To examine the effect of size and O₂ availability on the metabolic rate of *Musculium partumeium*, the following experimental design was utilized. The metabolic rates for three replicates of each of four size groups of clams were determined at six different levels of O₂ availability. This yielded 72 values of O₂ consumption.

Water with various levels of O₂ availability were prepared by mixing air-saturated, filtered pond water with nitrogen-saturated, filtered pond water in proportions of 1:0, 3:1, 1:1, 1:3, 1:7 and 0:1, thus giving six levels of O₂ availability. Because of the variability in the mixing process, changes in barometric pressure, etc., mixtures prepared on different days had varying Po₂s. The averages and ranges (in parentheses) of Po₂ values [measured by the azide-modified

Table 1. Summary of numbers, sizes and range of metabolic rates of *Musculium partumeium* utilized in this study. The coefficient of variation was calculated as the standard deviation as a percentage of the mean.

Experiment Number	Number of clams per bottle	Mean shell length	Mean ash-free dry weight (mg)	Range of metabolic rates ($\mu\text{l O}_2 \text{ AFDW}^{-1} \text{ hr}^{-1}$)	Mean coefficient of variation (%)
1	10	1.2	0.05	0.68-4.78	35.7
2	5	2.6	0.31	0.44-2.26	33.3
3	5	4.0	0.79	0.81-1.46	8.3
4	3	6.2	2.59	0.62-1.41	18.5

Winkler method (American Public Health Association, 1980)] were 140 mm Hg (117-166), 116 mm Hg (98-129), 93 mm Hg (75-109), 71 mm Hg (57-85), 58 mm Hg (42-72) and 41 mm Hg (27-50) for the mixtures (air-saturated water: nitrogen-saturated water) 1:0, 3:1, 1:1, 1:3, 1:7 and 0:1, respectively.

O₂ consumption of groups of 3-10 clams (depending on size) (Table 1) was measured after the specimens had been placed in 40 ml glass-stoppered bottles containing one of the pond water mixtures at 17°C. Control chambers (bottles with no clams) were analysed similarly as controls to correct for changes in O₂ concentration not directly attributable to clam metabolism. After 15-20 hr, the O₂ remaining in each chamber was measured with the azide-modified Winkler method. Duplicate 10.0 ml aliquots of water from each chamber were titrated with approximately 0.0025 N sodium thiosulfate (standardized with potassium bi-iodate) and a digital buret calibrated to 0.01 ml. Results were corrected for the volume of Winkler reagents used (0.5 ml each) in the fixation process. Additional details of this method can be found in Hornbach *et al.* (1983). Values as mg O₂ were converted to $\mu\text{l O}_2$ or Po₂ (mmHg) at STP with equations presented in Pierce *et al.* (1973). Ash free dry weights (AFDW) were determined for all groups of clams as the difference in weight after drying to constant weight at 100°C and ashing at 500°C.

RESULTS

Figure 1 shows the effect of O₂ availability on the rate of O₂ consumption for each of the four size categories of *Musculium partumeium* utilized in this study. Multiple regression analysis for each size class, with $\log_e(QO_2)$ ($\mu\text{l O}_2 \text{ mg AFDW}^{-1} \text{ hr}^{-1}$) as the dependent variable and Po₂ (O₂ tension mm Hg) and $\log_e(\text{AFDW})$ as the independent variables, showed that Po₂ significantly influenced QO₂ for the two largest size classes (4.0 mm - F=17.0, 1,16 df, p=0.0009; 6.2 mm - F=4.77, 1,17 df, p=0.045). For the two smaller size classes, there was no significant influence of Po₂ on QO₂ (1.2 mm - F=1.44, 1,17 df, p=0.248; 2.6 mm - F=1.35, 1,17, df, p=0.263). This lack of a statistically significant influence was due partially to the large degree of variability

in the O₂ consumption rates for these size groups of clams (Table 1). An overall regression analysis resulted in the following equation: $\log_e(QO_2) = -0.21 - 0.169 [\log_e(\text{AFDW})] + 0.003 (Po_2)$; $r^2=0.44$ (F=26.37, 2,70 df, p=0.0001). The standardized partial regression coefficients for $\log_e(\text{AFDW})$ and Po₂ are -0.66 and 0.28, indicating that AFDW had a higher degree of influence on metabolic rate than did O₂ availability.

Bayne (1971) derived an index for assessing the degree of influence of O₂ availability on O₂ consumption. If the consumption availability curve is hyperbolic, then $QO_2 = Po_2/k_1 + k_2 (Po_2)$. When Po₂/QO₂ is plotted against Po₂, a straight line is produced with k₁ the intercept and k₂ the slope. A high value of the ratio k₁/k₂ indicates a greater O₂-dependence of O₂ consumption. Regressions of these values were performed for each size class of clam to obtain estimates of k₁ and k₂. The results are shown in Table 2.

DISCUSSION

Several studies have shown that sphaeriid clams are capable of surviving various periods of anoxia (Juday, 1908;

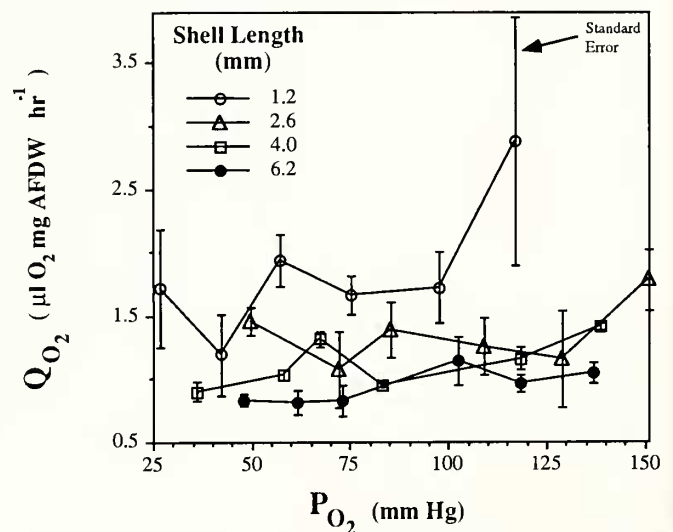


Fig. 1. Relationship between oxygen availability and oxygen consumption for four sizes of *Musculium partumeium*.

Table 2. Indices of oxygen dependence of metabolism for various sizes of *Musculium partumeium*. Indices are from the equation $P_{O_2}/Q_{O_2} = k_1 + k_2$ (P_{O_2}) and r^2 is the coefficient of determination.

Mean Shell Length (mm)	k_1	k_2	r^2	k_1/k_2
1.2	14.95	0.38	0.36	39.45
2.6	17.77	0.68	0.21	26.21
4.0	19.54	0.64	0.80	30.63
6.2	26.83	0.78	0.66	34.53

Eggleton, 1931; Thomas 1963, 1965; Gale, 1976; Holopainen and Jónasson, 1983; Way *et al.*, 1983; Holopainen, 1987). Nonetheless, few studies have examined the influence of O_2 availability on metabolism (Hornbach, 1985). Waite and Neufeld (1977) indicated that for the sphaeriid, *Sphaerium simile* (Say), there was little influence of O_2 availability on O_2 consumption over the range of 32-80 mm Hg P_{O_2} . Outside this range the influence of O_2 availability on O_2 consumption varied with temperature. Berg *et al.* (1962) found that O_2 consumption declined and reduced O_2 availability in *Pisidium casertanum* (Poli). Buchwalder (1983) found that *Musculium partumeium* and *M. lacustre* (Müller) displayed a dependence of O_2 consumption on O_2 availability and that this dependence varied seasonally. The greatest independence was found during periods of low O_2 availability and could help to explain how some sphaeriids can grow and reproduce under hypoxic conditions (Thomas, 1963, 1965; Way *et al.*, 1980). In this study I found that there is an effect of O_2 availability on O_2 consumption and that the effect appears to be size dependent (Fig. 1). There was considerable variability in the rates of consumption that were ascertained (Table 1). This high degree of variation could be due to behavioral differences in individuals (e.g. differences in locomotory or pumping activities) or differences in the reproductive condition of mature individuals. No attempt was made to observe individual variation in these parameters, however it was noted that in the vast majority of cases, all individuals were moving actively in the chambers, though it is unknown if this occurred continuously throughout the 15-20 hr experimental period. The fact that the variation in O_2 consumption is not significantly higher for brooding clams (6.2 mm size class - Table 1) suggests that reproductive condition does not alter significantly the variation noted by this technique.

Newell (1979), based on studies of marine bivalves by Bayne (1971, 1973) and Taylor and Brand (1975), claimed that small animals, with high weight-specific metabolic rates tend to have higher k_1/k_2 ratios than larger conspecific animals, indicating a decreased ability in smaller organisms to regulate O_2 consumption. Based on the k_1/k_2 values in Table 2, *Musculium partumeium* ≥ 2.6 mm appears to follow an opposite trend, that is, larger animals tend to have larger values

of k_1/k_2 indicating a greater independence of O_2 consumption on O_2 availability. *M. partumeium* in the 4.0 and 6.2 mm size classes are reproductively active and often contain extra-marsupial larvae (Hornbach *et al.*, 1980; unpubl data). It is possible that the larvae displayed a regulatory pattern from the adults and thus mask the response of the adult. For example, Burky and Burky (1976) have shown that over 50% of the O_2 consumption of adults of the sphaeriid, *Pisidium walkeri* Sterki, could be attributed to the respiration of incubating embryos.

Although the 1.2 mm clams had the highest k_1/k_2 ratio (Table 2), the r^2 of the relationship used to attain this value was low (Table 2). In fact, for clams of mean shell length 1.2 or 2.6 mm, there was no statistically significant influence of O_2 availability on O_2 consumption. This could indicate that these size clams are regulators. However, Table 1 indicates that one reason for the lack of a statistical relationship between O_2 availability and O_2 consumption in these size groups is due to the high individual variation in metabolic rates. The reasons for this variability are not known. However, in temporary ponds, many juvenile individuals do not grow during the summer. Apparently this lack of growth is required because only juveniles can overwinter in the dry pond (Way *et al.*, 1980; Hornbach and Childers, 1986). These overwintering juveniles grow to adults in the spring, reproduce and die during the summer. There is some evidence, however, that if temporary ponds remain wet through the fall, some portion of the juveniles produced in the spring and summer grow and reproduce in the fall (Hornbach *et al.*, 1980). There are varying genotypes in the population (McLeod *et al.*, 1981). It is possible that each is associated with a different metabolic rate as juvenile and this contributes to the observed variability (Table 1). The fact that juvenile *Musculium partumeium* must survive extremely long periods of anoxia, compared to many marine bivalves, could explain why their patterns of O_2 consumption dependency on O_2 availability do not follow those described by Newell (1979) for marine species.

The results of this study indicate that O_2 availability can influence O_2 consumption in *Musculium partumeium* but that the influence is size-specific. Furthermore it appears that the degree of variability in O_2 consumption is also influenced by size.

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